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Urban Nesting Adaptability of *Psenulus fuscipennis* (Hymenoptera: Crabronidae): First Record from Istanbul (Türkiye) and Insights from GLM-Based Abundance Modeling

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ABSTRACT

The fragmentation of urban green spaces in megacities such as Istanbul has raised concerns about the biodiversity of insects, particularly solitary hymenopterans. This study reports the first occurrence of *Psenulus fuscipennis* (Dahlbom, 1843) in Istanbul, Türkiye, and evaluates its nesting behaviour across an urbanisation gradient using artificial nest blocks. Twenty-five nest blocks, with entrance cavity diameters of 1.0, 0.7, and 0.4 cm, were monitored from March to September 2020. Nesting occurred exclusively in 0.4 cm cavities (n = 325 emergence events). The adults were identified morphologically, while the immature stages were confirmed by *COI* DNA barcoding. Importantly, the relationship between abundance and urbanisation depended on the analytical framework used. A non-parametric Kruskal-Wallis test comparing abundance among three urbanisation categories (low, semi, high) found no significant differences. However, a Generalised Linear Model (Poisson, log-link), which treated urbanisation as an ordinal predictor and controlled for habitat type (garden, park or rural), revealed a significant positive association between higher urbanisation intensity and abundance. This model also indicated higher abundance in rural locations than in gardens and lower abundance in parks. Thus, while categorical comparisons alone did not detect

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differences, model-based inference revealed a positive relationship between urbanisation and abundance once habitat variation was accounted for. Our results demonstrate that *P. fuscipennis* can utilise fragmented green spaces under urban pressure, with microhabitat (cavity size) remaining the primary constraint and the urbanisation signal likely reflecting unmeasured covariates rather than a general increase.

Keywords: Urban green spaces, Monitoring, DNA barcoding, Artificial nests.

INTRODUCTION

The urban expansion of densely populated cities such as Istanbul is increasingly recognised as a major factor contributing to biodiversity loss, particularly among hymenopteran insects. Previous studies (Zanette, Martin, & Ribeiro, 2005; Fenoglio, Rossetti, Videla, & Baselga, 2020) have demonstrated the degradation of urban green spaces (UGS), which are vital for the nesting and foraging of bees and wasps. The size and direct connection of green spaces to suburban areas are important for bee diversity, providing refuge zones in cities (Banaszak-Cibicka, Ratyńska, & Dylewski, 2016). With a population of 15 million people (according to 2020 data; TurkSat, 2021) within an area of 5,500 km² (Öztürk & Altay, 2015), Istanbul can be described as one of Türkiye's most densely populated cities. Consequently, Istanbul is experiencing pressure due to urbanisation and changes in land use (Güneralp, Tezer, & Albayrak, 2013).

Artificial nest blocks are an effective tool for monitoring Hymenoptera species in both urban and rural landscapes (Fortel, Henry, Guilbaud, Mouret, & Vaissiere, 2016; Geslin et al., 2020). They offer standardized sampling opportunities to evaluate nesting activity, abundance, and community composition of bees and wasps. Artificial nest blocks can also support and even enhance the presence of hymenopteran species (Fortel et al., 2016; González-Zamora, Hidalgo-Matas, & Corell-González, 2021). Therefore, artificial nest blocks are very important for the continuity of pollinator bees and other bee groups even in urbanized areas.

The Crabronidae family is one of the hymenopteran families (Hymenoptera: Apoidea) and is known as a member of the apoid wasps (Sann et al., 2018). It consists of five subfamilies: Astatinae, Bembicinae, Crabroninae, Pemphredoninae, and Philantinae (Rosa & Melo, 2020). The family plays a key role in maintaining ecological balance as a biological control agent, feeding on aphids, beetles, lepidopterans, hemipterans, cicadas, crickets and flies (Marchiori, 2023). There are more than 550 crabronid taxa in Türkiye (Gülmez, Çubuk, & Can, 2025).

Psenulus fuscipennis (Dahlbom, 1843) is a species of solitary wasp belonging to the Crabronidae family. It is widely distributed across Caucasia, Central Asia, the Far East, Europe and North Africa (Pulawski, 2025). It typically nests in pre-existing cavities, such as beetle burrows or artificial nests (Spooner, 1948; Taylor, Barthélémy, Chi, & Guénard, 2020). Adult *P. fuscipennis* feed on pollen, while their larvae feed on aphids and small hemipterans (Martynova & Fateryga, 2014). (Martynova & Fateryga, 2014). However, there is limited information on the nesting behaviour, habitat specificity and response to urbanisation of this species (Krombein, 1967; Van Lith, 1978; Matthews, 2001; Taylor et al., 2020). Therefore, monitoring studies are crucial for understanding the ecology of *P. fuscipennis*. Similarly, little is known about the distribution, nesting behaviour and ecology of *P. fuscipennis* in Türkiye (Yıldırım, Ljubomirov, & Lelej, 2014; Gülmez & Çubuk, 2018; Gülmez et al., 2025). *P. fuscipennis* has been recorded in Amasya, Ankara, Artvin, Çankırı, Kocaeli and Tokat (Gülmez & Çubuk, 2018; Gülmez et al., 2025). To our knowledge, this is the first record of *P. fuscipennis* in Istanbul.

The hypothesis that we tested in this study was whether *P. fuscipennis* has a particular nesting preference related to microhabitat characteristics such as cavity diameter and habitat type. We examined whether its abundance varied significantly along a gradient of urbanization.

MATERIALS AND METHODS

Field and Laboratory Studies

In March 2020, we deployed 25 artificial nest blocks at 25 distinct locations across Istanbul to monitor nesting preferences (Fig. 1). Each block contained a fixed set of pre-drilled cavities at three entrance diameters -1.0 cm (28 cavities), 0.7 cm (14 cavities), and 0.4 cm (18 cavities) -all with a uniform depth of 15 cm (Figs. 2a-b). This configuration yielded 60 cavities per block and 1,500 total cavities available for occupation (25 blocks × 60 cavities). The blocks were inspected bimonthly and subsequently collected in September 2020. All blocks were transferred to the Systematic Entomology Research Laboratory at Istanbul University for examination. After arrival, blocks were manually opened and their contents examined. Adult specimens were pinned and deposited in the insect collection, while larvae were separated for molecular identification. Both larval and adult individuals were photographed using a Leica M205 stereomicroscope (Fig. 3).

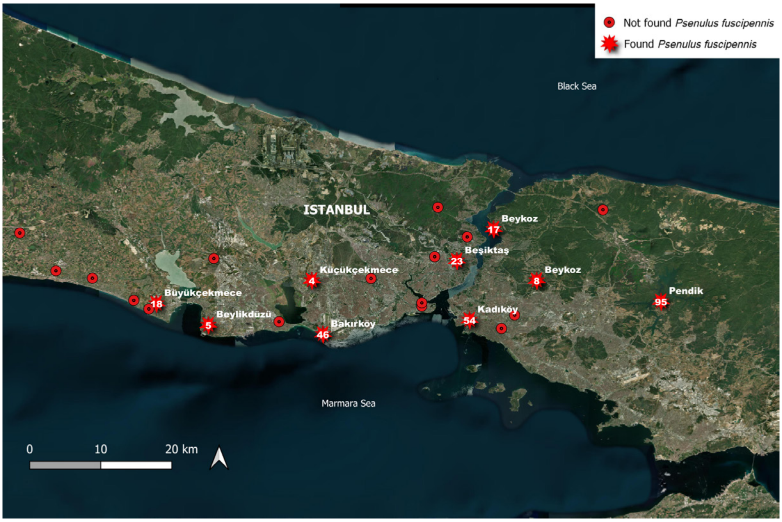

Figure 1. Distribution of artificial nest and number of individual of *P. fuscipennis*

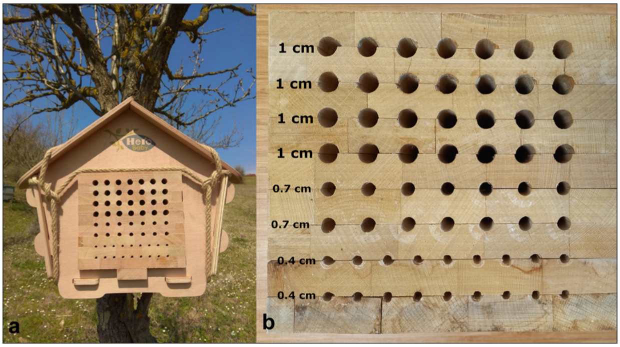

Figure 2. Artificial nest. a) Front view, b) width of bee nests.

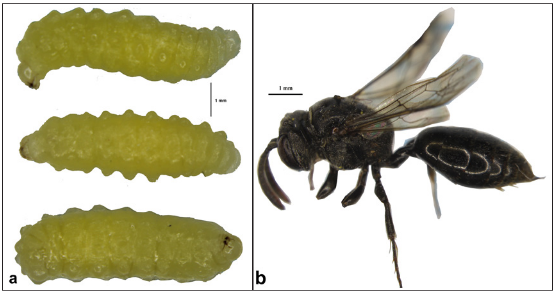

Figure 3. *P. fuscipennis.* a) Larvae, b) Adult.

Morphological Identifications and DNA Barcoding

Because most individuals recovered from the nest blocks were in pre-adult stages, species identification for these stages was performed exclusively via *COI* (cytochrome oxidase I) barcoding. Adult individuals were identified morphologically following de Beaumont (1937). Larvae were extracted from their cavities and preserved at −20 °C. Total genomic DNA was isolated using the Qiagen DNeasy Blood & Tissue Kit protocol. The partial *COI* region was amplified with primers LCO1490 and HCO2198 (Folmer et al., 1994; Table 1). PCR was run for 35 cycles (95 °C for 30 s, 48 °C for 30 s, and 72 °C for 45 s). Purified amplicons were sequenced using the Sanger dideoxy method. Sequences were aligned with MAFFT (Katoh et al., 2018) and trimmed manually. A maximum-likelihood phylogeny was inferred with PhyML 3.3.1 (Guindon et al., 2010) on NGPhylogeny (Lemoine et al., 2019). GenBank accession numbers for all samples are listed in Table 2.

Table 1. Primers used to partially amplify the *COI* gene region.

	Primer Sequence	Length	T_M	%GC
LCO1490	5’-GGTCAACAAATCATAAAGATATTGG-3’	25 mer	59.2	32
HCO2198	5’-TAAACTTCAGGGTGACCAAAAAATCA- 3’	26 mer	61.7	34

Table 2. Species and Accession Numbers of the samples.

Species	Accession Number	Sample
Psenulus fuscipennis	OQ781230	Used in the study
Psenulus fuscipennis	OQ781244	Used in the study
Psenulus fuscipennis	OQ781256	Used in the study
Psenulus fuscipennis	OQ781261	Used in the study
Psenulus fuscipennis	OQ781234	Used in the study
Psenulus fuscipennis	OQ781246	Used in the study
Psenulus fuscipennis	OQ781227	Used in the study
Psenulus fuscipennis	OQ781245	Used in the study
Psenulus fuscipennis	MH609270.1	GenBank
Psenulus fuscipennis	MH610357.1	GenBank
Psenulus fuscipennis	MH610880.1	GenBank
Psenulus fuscipennis	MZ623555.1	GenBank
Psenulus fuscipennis	MZ628899.1	GenBank
Psenulus fuscipennis	MH611037.1	GenBank
Psenulus fuscipennis	MH609429.1	GenBank
Psenulus fuscipennis	KP696365.1	GenBank
Psenulus laevigatus	HQ947700.1	GenBank
Psenulus laevigatus	HQ947703.1	GenBank
Psenulus laevigatus	MH610204.1	GenBank
Psenulus pallipes	HQ947694.1	GenBank
Psenulus pallipes	HQ947695.1	GenBank
Psenulus pallipes	HQ947701.1	GenBank
Anthidium florentinum	KJ839553.1	GenBank
Anthidium florentinum	KJ837808.1	GenBank
Anthidium florentinum	MT869110.1	GenBank
Apis mellifera	MT745904.1	GenBank
Apis mellifera	NC_051932.1	GenBank
Apis mellifera	MT745907.1	GenBank

Statistical Analysis

To assess whether the number of individuals significantly differed among urbanization levels (Low, Semi, High), a non-parametric Kruskal-Wallis test was applied. This test was selected to determine whether urbanization intensity had a statistically significant effect on individual abundance. All the statistical analyses were performed by using R software version 4.2.3 (R Core Team, 2022) and the test was performed with the kruskal.test() function. Also, based on a Poisson distribution fitted with a log-link function, a Generalized Linear Model (GLM) was used to examine the effects of habitat type and urbanization level on the number of individuals occupying three different diameter cavities. The response variable was the count of individuals per location, and the explanatory variables included urbanization level (treated as an ordinal numeric variable: Low = 1, Semi = 2, High = 3) and habitat type (categorical: Garden, Park, Rural). The level of urbanization was determined depending on the urbanization density in the area where the artificial nest blocks are located (TurkSat, 2021; Kaya, 2024). Model fitting was performed in R software version 4.2.3 (R Core Team, 2022) using the glm() function from the base stats package (Dobson & Barnett, 2018). Significance of predictor variables was evaluated using Wald chi-square tests, and model outputs were interpreted at a 0.05 significance level. We did not perform an omnibus among-location Kruskal-Wallis test on raw counts because each location contributed a single observation and our inferential focus was on gradients of habitat and urbanization; among-location heterogeneity is therefore addressed within the GLM framework. The results were visualized using ggplot2 by comparing observed and predicted values.

RESULTS

Throughout the monitoring period, nesting activity was observed exclusively in the 0.4 cm diameter cavities. No occupation was recorded in the 0.7 cm and 1.0 cm diameter cavities across any of the sampling locations. A total of 325 individuals emerged from the 0.4 cm cavities, with considerable variation in abundance across locations. The highest number of individuals (n = 107) was recorded in a rural location (Pendik) (Fig. 1), while the remaining locations - regardless of urbanization level - showed lower and more variable occupation rates (Fig. 4). Some high-urbanization parks and gardens yielded relatively high counts (e.g., 63 and 51 individuals, respectively).

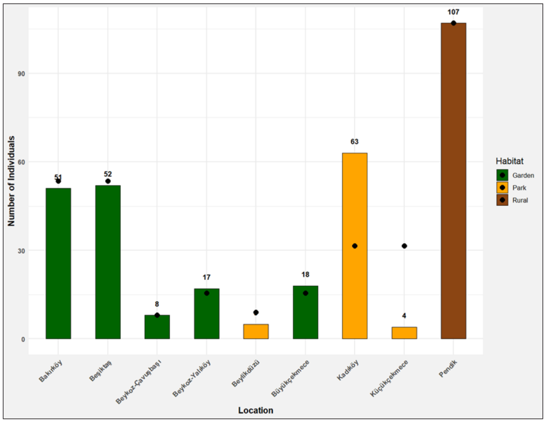

Figure 4. Observed and predicted abundance of *Psenulus fuscipennis* across sampling locations. Colored bars show observed counts in 0.4 cm cavities, categorized by habitat type (Garden, Park, Rural). Black points are predictions from a Poisson GLM (log-link) including urbanization (ordinal) and habitat as predictors. The figure provides an illustrative comparison between raw counts and model predictions; visual similarity is descriptive and not a formal assessment of model fit. Inference about habitat and urbanization effects relies on GLM coefficients and diagnostics rather than bar-point agreement.

COI analysis on larvae obtained from eight locations and morphological identification on adult individuals from one location revealed the species *P. fuscipennis* (Fig. 3). As a result of models, GTR with gamma-distributed rate was selected as the best substitution model for *COI* gene region by analysis based on Maximum Likelihood in PhyML Version 3.3.1 (Guindon et al., 2010). It is shown in the phylogenetic tree that the studied specimens match *P. fuscipennis* (Fig. 5).

To evaluate whether urbanization level had an effect on the abundance of individuals using 0.4 cm diameter cavities, a Kruskal-Wallis test was conducted. The test revealed no statistically significant differences among the three urbanization categories—Low, Semi, and High (H = 1.11, p = 0.574).

To further examine the influence of habitat type and urbanization level on the abundance of *P. fuscipennis* individuals nesting in 0.4 cm cavities, we fitted a Generalized Linear Model (Poisson, log-link) that treated urbanization as an ordinal predictor (Low=1, Semi=2, High=3) and included habitat type as a categorical factor (Garden, Park, Rural). The model showed significant habitat effects (Rural > Garden,

$p < 0.001$; Park < Garden, $p = 0.029$) and a significant positive effect of urbanization intensity on abundance ($p < 0.001$). Thus, while categorical comparisons alone (Kruskal-Wallis) did not reveal differences among urbanization groups, the GLM uncovered a positive abundance-urbanization relationship once habitat was controlled. Figure 4 presents descriptive site-level counts (bars) and GLM predictions (points) (Table 3). This qualitative agreement is not a formal test of model fit; therefore, we refrain from inferring explanatory power from visual inspection alone. Model performance was evaluated using residual diagnostics, dispersion checks, and information criteria, and inference about predictors derives from the GLM coefficients and associated tests.

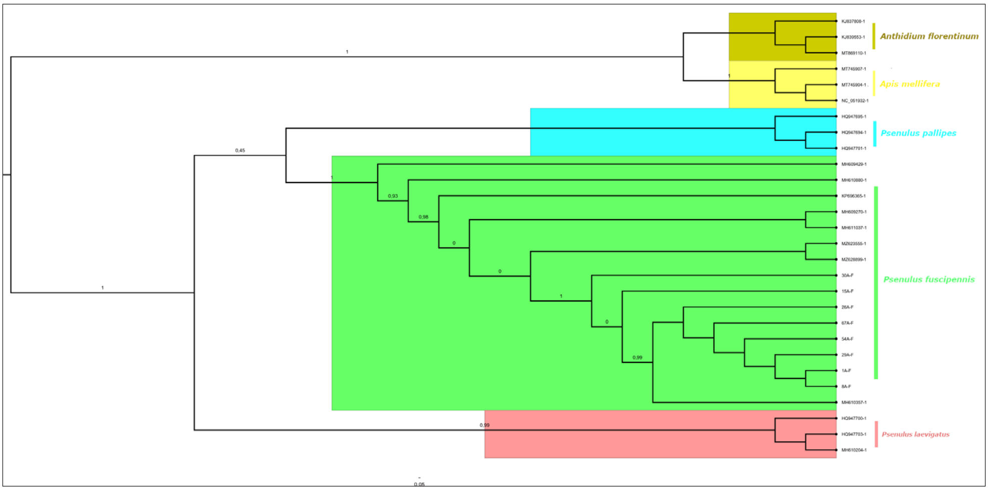

Figure 5. The phylogenetic tree based on the Maximum Likelihood using PhyML of *P. fuscipennis*.

Table 3. Wald chi-square test results from the Generalized Linear Model (GLM) with Poisson distribution evaluating the effects of urbanization level and habitat type on the abundance of *Psenulus fuscipennis* in 0.4 cm cavities. The table includes estimated coefficients, standard errors, z-values, and *p*-values. Significant predictors are shown in bold ($p < 0.05$). Reference categories: low urbanization and garden habitat.

Predictor	Estimate	Std. Error	z value	p-value	95% CI (Lower-Upper)
(Intercept)	2.079	0.354	5.88	**< 0.001**	1.39 - 2.77
Urbanization (Semi)	0.659	0.389	1.69	0.090	-0.10 - 1.42
Urbanization (High)	1.901	0.366	5.20	**< 0.001**	1.18 - 2.62
Habitat: Park	-0.532	0.146	-3.63	**0.00028**	-0.82 - -0.24
Habitat: Rural	2.593	0.367	7.08	**< 0.001**	1.87 - 3.31

DISCUSSION

This study reports the first occurrence of *Psenulus fuscipennis* in Istanbul, thereby providing a faunistic record for the city and expanding its known distribution in the country. The successful detection of *P. fuscipennis* through artificial nest blocks emphasizes both the species' adaptability and the utility of standardized cavity-nesting surveys in urban ecological monitoring.

The Kruskal-Wallis test did not detect significant differences among urbanization categories because it compares group medians without accounting for other predictors. The GLM, which modeled counts with a Poisson error, treated urbanization as an ordered gradient and controlled for habitat type, and therefore detected a significant positive association between higher urbanization intensity and abundance while also showing habitat effects (Rural > Garden; Park < Garden). This clarifies that apparent “no difference” across categories does not contradict a positive gradient effect once habitat structure is included. Accordingly, Figure 4 should be interpreted as illustrative rather than confirmatory; our conclusions about habitat and urbanization effects rely on the fitted GLM and its diagnostics, not on visual comparisons of raw counts to fitted values at individual locations.

As the species showed no nesting activity in the 0.7 cm and 1.0 cm cavities, the exclusive occupation of 0.4 cm diameter cavities indicates a distinct preference or morphological constraint. The results are consistent with previous studies showing that cavity-nesting Hymenoptera have a strong preference for entrance diameters based on body size compatibility, thermoregulation, or protection from predators and competitors (Roulston & Cane, 2000; Du et al., 2025; Brozoski et al., 2023). While *P. fuscipennis* is not considered a primary pollinator, its frequent visits to flowering plants may contribute incidentally to pollination, and its provisioning behavior targeting aphids suggests potential for regulating pest populations (Martynova & Fateryga, 2014).

Although the highest individual count was observed in a rural location (Fig. 4), no statistically significant difference was found among urbanization levels. Individuals were found nesting not only in low-disturbance rural environments but also in parks and gardens located in semi- and high-urbanization zones. This broad spatial distribution suggests that *P. fuscipennis* is capable of tolerating and adapting to various degrees of urban intensity, provided that suitable nesting substrates are available. The presence of the species in fragmented green areas across the city implies a level of ecological plasticity that may facilitate its persistence in highly modified urban ecosystems.

The results of the Generalized Linear Model (GLM) and associated Wald chi-square tests provide strong evidence that both habitat type and urbanization level significantly influence the nesting abundance of *P. fuscipennis* in artificial cavities. The GLM model indicated a substantially higher number of individuals in rural locations compared to gardens, while park habitats supported significantly fewer individuals (Table 3). These findings are consistent with the general expectation that rural environments offer more stable and diverse floral and nesting resources for cavity-nesting hymenopterans. Interestingly, only high levels of urbanization were associated with a significant increase in nesting abundance, whereas medium levels did not show a statistically significant effect. Collectively, our results do not imply that urbanization per se increases *P. fuscipennis* abundance. Given that larvae feed on aphids and small hemipterans (see Martynova & Fateryga, 2014), the positive urbanization coefficient in the GLM could reflect unmeasured resource covariates in some highly urbanized locations (e.g., local prey availability or microhabitat features) once habitat type is controlled. However, we did not measure these variables; therefore we treat this as a plausible pathway rather than a demonstrated mechanism. Notably, the highest abundance occurred at a rural location and categorical tests did not detect group differences.

The significance of both habitat and urbanization predictors in the Wald chi-square test further confirms the robustness of the GLM model. The test results indicate that the variation in individual counts is not due to chance alone, reinforcing the ecological relevance of habitat structure and anthropogenic pressure gradients in shaping solitary wasp nesting behavior. These findings suggest that while *P. fuscipennis* demonstrates a degree of adaptability to urbanized environments, its highest nesting success still occurs in less disturbed, rural conditions. Thus, although the species can persist in highly urbanized areas where appropriate microhabitats and prey are available, preserving semi-natural and rural habitats remains essential for sustaining optimal nesting behavior. We also note that our inference is based on a single-season, cross-sectional design; future replicated studies quantifying prey availability, floral resources, and microclimate will be necessary to test the mechanisms underlying these patterns.

Although the Kruskal-Wallis test did not detect a statistically significant difference in nesting abundance among the sampling locations, the Generalized Linear Model (GLM) analysis revealed that both habitat type and urbanization level had significant effects on individual abundance ($p < 0.001$ for rural and park habitats; $p < 0.001$ for high urbanization). However, medium urbanization did not have a statistically significant effect. This discrepancy is likely due to the differences in the analytical scope of the two methods. As a non-parametric rank-based test, Kruskal-Wallis only evaluates overall differences between the medians of independent groups and does not account for the simultaneous influence of multiple predictors. GLM, by contrast, models count data with distributional assumptions (in this case, Poisson) and evaluates the unique contribution of each explanatory variable while controlling for others. Therefore, GLM is more sensitive in detecting complex ecological patterns, particularly in datasets with interacting gradients such as habitat type and urbanization intensity. Thus, GLM results provide a better understanding of factors influencing nesting behavior in *P. fuscipennis*.

Overall, the findings highlight the importance of microhabitat characteristics—such as cavity size—over broader land-use gradients in determining solitary wasp nesting success. Moreover, the study reinforces the value of artificial nest surveys in assessing hymenopteran diversity and behavior across urban environments, especially in regions where natural nesting locations are limited due to habitat fragmentation.

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